

We claim:

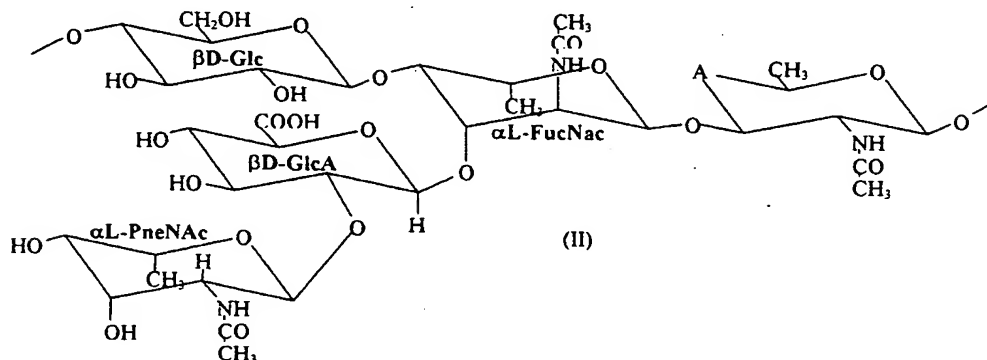
1. A pneumococcus type 5 capsular polysaccharide which is aminated on the terminal aldehyde group and which exhibits (i) a carbon ( $^{13}\text{C}$ ) NMR spectrum lacking a resonance signal between 13 and 14 ppm inclusive; (ii) an HPAEC-PAD chromatogram obtained by elution from a Carbopac<sup>TM</sup> PA10 column in an 18 mM sodium hydroxide solution at a flow rate of 1 ml/min for 15 min of monosaccharides derived from hydrolysis of said polysaccharide, wherein said chromatogram lacks a peak between fucosamine and pneumosamine peaks; or (iii) both.
2. The polysaccharide according to Claim 1, which exhibits:
  - (i) a carbon NMR spectrum which comprises a resonance signal between 11.5 and 12.5 ppm, inclusive, characteristic of a Sug compound, and a resonance signal located between 17 and 18 ppm inclusive, characteristic of N-acetylated quinovosamine, the intensity of which is less in comparison with the resonance signal located between 17 and 18 ppm, inclusive, in the ( $^{13}\text{C}$ ) NMR spectrum of a pneumococcus type 5 capsular polysaccharide obtained after reductive amination in the presence of sodium cyanoborohydride at pH 8 for 48 hours; or
  - (ii) an HPAEC-PAD chromatogram obtained under the conditions specified in Claim 1, which comprises a peak located immediately after the pneumosamine peak, characteristic of quinovosamine, the intensity of which is less in comparison with the equivalent peak in the HPAEC-PAD chromatogram of a pneumococcus type 5 capsular polysaccharide obtained after reductive amination in the presence of sodium cyanoborohydride at pH 8 for 48 hours; or

- (iii) both.
3. The polysaccharide according to Claim 1 which exhibits (i) a carbon NMR spectrum entirely lacking a resonance signal between 17 and 18 ppm; (ii) an HPAEC-PAD chromatogram obtained according to the conditions of claim 1 lacking a quinovosamine peak, the peak observed with the polysaccharide aminated according to a conventional amination method being reduced so as to be no more than a simple shoulder of the preceding peak (pneumosamine peak) ; or (iii) both.
4. The aminated polysaccharide according to Claim 1, which exhibits:
- (i) a carbon NMR spectrum lacking a resonance signal between 11.5 and 12.5 ppm, inclusive, characteristic of a Sug compound, which comprises a resonance signal located between 17 and 18 ppm inclusive, characteristic of N-acetylated quinovosamine, the intensity of which is increased in comparison with the resonance signal located between 17 and 18 ppm, inclusive, in the ( $^{13}\text{C}$ ) NMR spectrum of a pneumococcus type 5 capsular polysaccharide obtained after reductive amination in the presence of sodium cyanoborohydride at pH 8 for 48 hours; or
  - (ii) an HPAEC-PAD chromatogram obtained under the specified in Claim 1 which comprises a peak located immediately after the pneumosamine peak, characteristic of quinovosamine, the intensity of which is increased in comparison with the equivalent peak in the HPAEC-PAD chromatogram of a pneumococcus type 5 capsular polysaccharide obtained after reductive amination in the presence

of sodium cyanoborohydride at pH 8 for 48 hours ;  
or

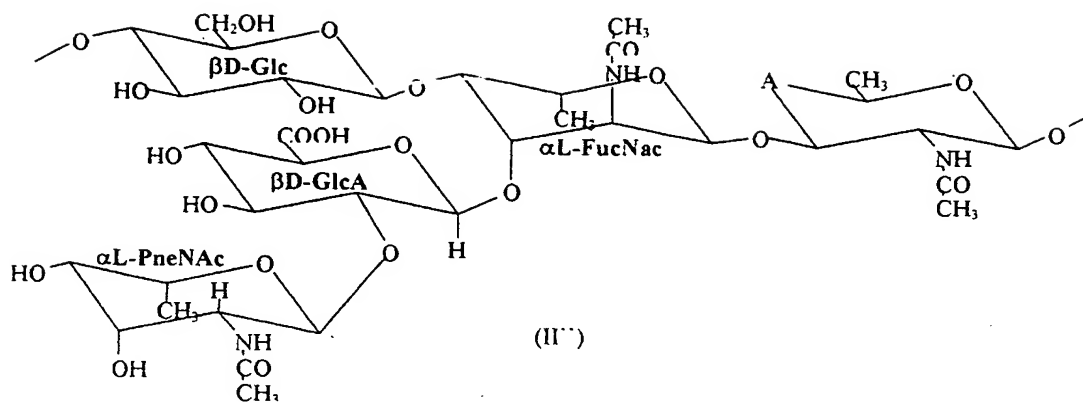
(iii) both.

5. A pneumococcus type 5 capsular polysaccharide which is aminated on the terminal aldehyde group, consisting of repeating units, at least 85% of the repeating units of which correspond to formula (II)



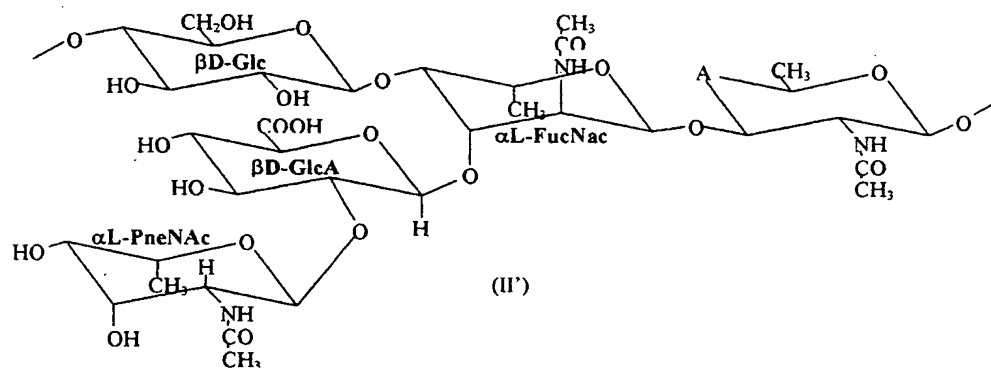
in which A is independently and randomly C=O or CHOH.

6. The polysaccharide according to Claim 5 in which at least 90% of the repeating units correspond to formula (II).
7. The polysaccharide according to Claim 6 in which at least 95% of the repeating units correspond to formula (II).
8. The polysaccharide according to Claim 5 in which at least 95% of the repeating units corresponding to formula (II) correspond to formula II"



in which A is CHOH.

9. The polysaccharide according to Claim 5 in which 85 to 95% of the repeating units corresponding to formula (II) correspond to formula II'



in which A is C=O.

10. The conjugate in which a polysaccharide according to Claim 1 is coupled to a carrier polypeptide (P).
11. A method for producing an aminated pneumococcus type 5 capsular polysaccharide, wherein the polysaccharide is subjected to a reductive amination in the presence of a reducing agent selective for a Schiff base at a pH of 4 to 6.5 for a period not exceeding 4 hours.

12. The method according to Claim 11 in which the polysaccharide is subjected to a reductive amination at a pH of 5 to 6.
13. The method according to Claim 11 in which the polysaccharide is subjected to a reductive amination for a period not exceeding 2 hours.
14. The method according to Claim 11 in which the reducing agent selective for a Schiff base is cyanoborohydride or pyridine borane complex.
15. A method for producing an aminated pneumococcus type 5 capsular polysaccharide, according to which (i) the polysaccharide is reacted with an agent for reducing a ketone function, (ii) the reduced polysaccharide is fragmented, and (iii) the reduced and fragmented polysaccharide is subjected to a reductive amination.
16. The method according to Claim 15 in which the polysaccharide which is reacted with the agent capable of reducing a ketone function is in native form.
17. The method according to Claim 15 in which the agent capable of reducing a ketone function is  $\text{NaBH}_4$ .
18. The method according to Claim 15 in which the reduced polysaccharide is fragmented by oxidative free-radical depolymerization.
19. A method for producing a conjugate of formula  $\text{Ps-CH}_2\text{-NH-P}$ , according to which a method according to Claim 11 is used, wherein the pneumococcus type 5 capsular polysaccharide (Ps) is coupled by reductive amination to a carrier polypeptide (P) in the presence of a reducing agent selective for a Schiff base at a pH of 4 to 6.5 for a period not exceeding 4 hours.

20. A method for producing a conjugate of formula  $\text{Ps-CH}_2\text{-NH-P}$ , according to which a method according to Claim 15 is used, in which the reduced and fragmented pneumococcus type 5 capsular polysaccharide (Ps) is coupled by reductive amination with a carrier polypeptide (P).
21. A method for producing a conjugate of formula  $\text{Ps-CH}_2\text{-NH-L-P}$ , according to which:
- (i) the method according to Claim 11 is used, wherein the pneumococcus type 5 capsular polysaccharide (Ps) is coupled by reductive amination to a linking agent (L) having at least one free amine function so as to form an aminated and activated polysaccharide of formula  $\text{Ps-CH}_2\text{-NH-L}$ , and the activated polysaccharide is coupled to a carrier polypeptide (P) in order to obtain the conjugate of formula  $\text{Ps-CH}_2\text{-NH-L-P}$ ; or, alternatively,
  - (ii) the method according to Claim 11 is used, wherein the pneumococcus type 5 capsular polysaccharide (Ps) is coupled by reductive amination to an activated carrier polypeptide of formula  $\text{L-P}$ , wherein L is a linking agent having at least one free amine function, in order to obtain the conjugate of formula  $\text{Ps-CH}_2\text{-NH-L-P}$ .
22. A method for producing a conjugate of formula  $\text{Ps-CH}_2\text{-NH-L-P}$ , in which:
- (i) (a) a method according to Claim 15 is used, wherein the reduced and fragmented pneumococcus type 5 capsular polysaccharide (Ps) is coupled by reductive amination to a linking agent (L) having at least one free amine function so as to form an aminated and activated polysaccharide of formula  $\text{Ps-CH}_2\text{-NH-L}$ , and (b) the activated polysaccharide is coupled to a carrier polypeptide (P) in order to obtain the conjugate of formula  $\text{Ps-CH}_2\text{-NH-L-P}$ ; or, alternatively,

- (ii) the method according to Claim 15 is used, wherein the reduced and fragmented pneumococcus type 5 capsular polysaccharide (Ps) is coupled by reductive amination to an activated carrier polypeptide of formula L-P, wherein L is a linking agent having at least one free amine function, in order to obtain the conjugate of formula  $\text{Ps-CH}_2\text{-NH-L-P}$ .
23. A method for producing a conjugate of formula  $\text{Ps-CH}_2\text{-NH-S-L'-P}$ , in which:
- (i) the method according to Claim 11 is used, wherein the pneumococcus type 5 capsular polysaccharide (Ps) is coupled by reductive amination to a spacer (S) having at least one free amine function, so as to form an aminated and derivatized polysaccharide of formula  $\text{Ps-CH}_2\text{-NH-S}$ , and
- (ii) (a) the derivatized polysaccharide is coupled with a linking agent (L'), in order to obtain an activated polysaccharide of formula  $\text{Ps-CH}_2\text{-NH-S-L'}$ , then the activated polysaccharide is coupled with a carrier polypeptide (P), in order to obtain the conjugate of formula  $\text{Ps-CH}_2\text{-NH-S-L'-P}$ ; or, alternatively,
- (b) the derivatized polysaccharide is coupled with an activated carrier polypeptide of formula L'-P, in which L' is a linking agent, in order to obtain the conjugate of formula  $\text{Ps-CH}_2\text{-NH-S-L'-P}$ .
24. A method for producing a conjugate of formula  $\text{Ps-CH}_2\text{-NH-S-L'-P}$ , in which:
- (i) the method according to Claim 15 is used, wherein the reduced and fragmented pneumococcus type 5 capsular polysaccharide (Ps) is coupled by reductive amination to a spacer (S) having at least one free amine function so as to form an aminated and derivatized polysaccharide of formula  $\text{Ps-CH}_2\text{-NH-S}$ , and

- (ii) (a) the derivatized polysaccharide is coupled with a linking agent ( $L'$ ) in order to obtain an activated polysaccharide of formula  $Ps-CH_2-NH-S-L'$ , then the activated polysaccharide is coupled with a carrier polypeptide ( $P$ ), in order to obtain the conjugate of formula  $Ps-CH_2-NH-S-L'-P$ ; or, alternatively,
- (b) the derivatized polysaccharide is coupled with an activated carrier polypeptide of formula  $L'-P$ , wherein  $L'$  is a linking agent, in order to obtain the conjugate of formula  $Ps-CH_2-NH-S-L'-P$ .
25. The method according to Claim 19 , wherein the carrier polypeptide  $P$  is diphtheria toxoid or tetanus toxoid.
26. The method according to Claim 21 , wherein the linking agent ( $L$ ) is a compound of formula (XII)  $R_1-A-R_2$ , in which:
- $A$  denotes an aliphatic or aromatic chain or a mixed aliphatic and aromatic chain, which may be substituted or unsubstituted;
- $R_1$  denotes a primary amine or a chemical radical carrying a primary amine; and
- $R_2$  denotes a functional group capable of reacting with a carbonyl, thiol or amine group.
27. The method according to Claim 26, wherein the linking agent ( $L$ ) is an alkyl dihydrazide or a diaminoalkyl.
28. The method according to Claim 23 , wherein the spacer  $S$  is an aminothiols and the linking agent  $L'$  is a succinimidylmaleimidylalkyl.
29. The method according to Claim 23 , wherein the spacer  $S$  is a diaminoalkyl or a dihydrazide, and the linking agent  $L'$  is chosen from disuccinimidylalkyl or succinimidylmaleimidoalkyl compounds of formula (XIV)  $R_3-B-R_4$  in which  $B$  is an alkyl group,  $R_3$  is a succinimidyl group and  $R_4$  is a succinimidyl or maleimido group.



30. A pharmaceutical composition comprising a conjugate according to Claim 10.
31. A pharmaceutical composition comprising a conjugate obtained using the method according to Claim 19.